

Hindbrain mineralocorticoid mechanisms on sodium appetite

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Formenti S, Bassi M, Nakamura NB, Schoorlemmer GHM, Menani JV, Colombari E. Hindbrain mineralocorticoid mechanisms on sodium appetite. *Am J Physiol Regul Integr Comp Physiol* 304: R252–R259, 2013. First published November 28, 2012; doi:10.1152/ajpregu.00385.2011.—Aldosterone acting on the brain stimulates sodium appetite and sympathetic activity by mechanisms that are still not completely clear. In the present study, we investigated the effects of chronic infusion of aldosterone and acute injection of the mineralocorticoid receptor (MR) antagonist RU 28318 into the fourth ventricle (4th V) on sodium appetite. Male Wistar rats (280–350 g) with a stainless-steel cannula in either the 4th V or lateral ventricle (LV) were used. Daily intake of 0.3 M NaCl increased to 46 ± 15 and 130 ± 6 ml/24 h after 6 days of infusion of 10 and 100 ng/h of aldosterone into the 4th V (intake with vehicle infusion: 2 ± 1 ml/24 h). Water intake fell slightly and not consistently, and food intake was not affected by aldosterone. Sodium appetite induced by diuretic (furosemide) combined with 24 h of a low-sodium diet fell from 12 ± 1.7 ml/2 h to 5.6 ± 0.8 ml/2 h after injection of the MR antagonist RU 28318 (100 ng/2 μ l) into the 4th V. RU 28318 also reduced the intake of 0.3 M NaCl induced by 9 days of a low-sodium diet from 9.5 ± 2.6 ml/2 h to 1.2 ± 0.6 ml/2 h. Infusion of 100 or 500 ng/h of aldosterone into the LV did not affect daily intake of 0.3 M NaCl. The results are functional evidence that aldosterone acting on MR in the hindbrain activates a powerful mechanism involved in the control of sodium appetite.

aldosterone; mineralocorticoid receptor; sodium ingestion; sodium depletion; RU 28318

SODIUM IS THE MAIN ELECTROLYTE involved in the control of extracellular fluid osmolarity and volume and, consequently, affects blood pressure in physiological and pathological conditions. Sodium appetite, the behavior that drives animals and humans to ingest sodium, is stimulated by body sodium deficiency, a condition that usually activates the renin-angiotensin-aldosterone system (3, 14, 32, 43, 50, 57).

ANG II and aldosterone, the two main hormones produced by the renin-angiotensin-aldosterone system, act in the brain in a synergistic manner to stimulate sodium appetite (13, 15, 17, 47, 50). It is well established that ANG II induces water and sodium intake by acting on AT₁ receptors located in the lamina terminalis, in particular, in the organum vasculosum of the lamina terminalis and the subfornical organ (4, 8, 30, 34). The mineralocorticoid hormone aldosterone is secreted by the adrenal cortex in response to increased level of plasma ANG II, usually in conditions of fluid and sodium deficiency. Aldosterone acts on the kidney to reduce renal sodium excretion and on the brain to stimulate sodium

intake (37, 41, 45, 46, 47, 49, 59, 60). In addition, it has been suggested that aldosterone can stimulate mineralocorticoid receptors (MR) in the paraventricular hypothalamic nucleus, leading to increased sympathetic activity that may cause cardiovascular diseases like hypertension (5, 18, 24, 25, 26, 27).

Systemic administration of aldosterone or DOCA induces sodium appetite, a response abolished or reduced by electrolytic lesions or injections of antisense oligodeoxynucleotides against MR into the amygdala (19, 37, 45, 46, 49, 60). Injections of aldosterone or DOCA into the amygdala also induce intake of hypertonic NaCl in next 15–30 min (46). Aldosterone-sensitive neurons that might be involved in the control of sodium intake have also been identified in the nucleus of the solitary tract (NTS), close to the fourth ventricle (4th V) (20, 21). These neurons, referred to as HSD2 neurons, coexpress MR and the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (HSD2) (21). The HSD2 enzyme inactivates the endogenous glucocorticoids and, thus, allows only aldosterone to access the MR (36, 38, 39). The HSD2 neurons are activated in sodium-depleted rats and are deactivated by sodium ingestion, which suggests that they play a role in some aspects of sodium homeostasis (20, 21, 23).

Previous studies have investigated almost exclusively the induction of sodium appetite by facilitatory signals produced the action of ANG II or mineralocorticoids in the forebrain areas and have assumed that the role of the hindbrain in sodium appetite is mainly in the processing of sensory signals arising from the periphery that inhibit sodium appetite (1, 4, 6, 7, 12, 16, 28, 29, 35, 37, 47, 49, 53, 55, 60). Considering the importance of aldosterone and the immunohistochemical evidence such HSD2 neurons in the NTS may participate in the control of sodium appetite, in the present study, we tested whether sodium intake is affected by chronic infusion of aldosterone into the 4th V. Infusions were made with both a syringe pump and osmotic minipumps. We also tested whether bolus injections of the specific MR antagonist RU 28318 (31) into the 4th V inhibits intake of 0.3 M NaCl. Finally, the effects of aldosterone infusions and aldosterone antagonist injections into the lateral ventricle on sodium intake were also tested.

MATERIALS AND METHODS

Animals

Male Wistar Hannover rats weighing 280 to 350 g were housed individually and maintained in a room with controlled temperature ($22 \pm 2^\circ\text{C}$), humidity (40 to 60%), and a 12:12-h light-dark cycle. Animals had free access to tap water, 0.3 M NaCl and regular rodent chow (Nuvilab CR1; Colombo, PR, Brazil), except when specified in the protocol. All of the experiments were done in accordance with the Brazilian Society for Neuroscience and Behavior Guidelines for Animal Experimentation and were approved by the Animal Experimentation Ethics Committee of the

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Federal University of São Paulo School of Medicine (protocol no. 1805/07).

Brain Surgery

Rats were anesthetized with ketamine (100 mg/kg body wt ip) and xylazine (10 mg/kg body wt ip) (Vetbrands, Jacareí, SP, Brazil) and placed in a stereotaxic apparatus (Kopf, Tujunga, CA, USA). The skull was positioned with bregma and lambda at the same horizontal level. A 23-gauge stainless-steel guide cannula was implanted in the 4th V or lateral ventricle (LV). For the 4th V, the guide cannula was positioned on the midline, 12.8 mm caudal to bregma, and 6.1 mm below the surface of the skull. For the LV, the cannula was placed 0.3 mm caudal to bregma, 1.5 mm lateral to the midline, and 3.1 mm below the surface of the skull (40). The guide cannulas were fixed to the skull with stainless-steel screws and dental acrylic resin. An obturator closed the cannula except during injections or infusions. At the end of the surgery, each rat received a prophylactic dose of veterinarian antibiotic (Pentabiotico, 0.2 ml, 1:200,000 IU; Fort-Dodge, Campinas, SP, Brazil) intramuscularly. After surgery, the animals were housed individually and allowed to recover for 8 to 10 days before the start of experiments.

Correct placement of LV cannulas was verified 5 days after surgery by measuring the dipsogenic response to an intracerebroventricular injection of 100 ng of ANG II. Only rats that drank at least 5 ml of water in the 10 min after injection were used to study effects of aldosterone infusion into the LV.

Intracerebroventricular Infusions

To make tethered infusions, the obturator was removed from the guide cannula and a 30-gauge injector, 2 mm longer than the guide cannula, was introduced into the guide cannula. The injector was connected with Teflon tubing to a swivel joint that was mounted above the cage on a counterbalanced arm, allowing free movement to the rat. A stainless-steel spring protected the tubing from damage. The swivel joint was connected with Teflon tubing to a 100- μ l "airtight" Hamilton syringe (1710; Hamilton, Reno, NV) that was mounted on a syringe pump (YA-12; Braintree Scientific, Braintree, MA). Infusions were 2 μ l/h for 6 days.

Untethered infusions were made with osmotic minipumps (model 2001; Durect, Cupertino, CA). Pumps were filled with the solution to be infused and incubated in saline for at least 24 h at room temperature. Rats were anesthetized with 1% halothane. The pump was implanted under the skin in the scapular region and connected with polyethylene tubing to an injector cannula that was placed in the guide cannula. The injector cannula was made of 30-gauge stainless-steel tubing bent at a 90° angle, and protruded 2 mm beyond the tip of the guide cannula. Infusions were 2 μ l/h for 7 days.

Intracerebroventricular Bolus Injection

Bolus injections were made with a 10- μ l syringe (Hamilton, Reno, NV) connected with polyethylene (PE)-10 tubing to an injector made

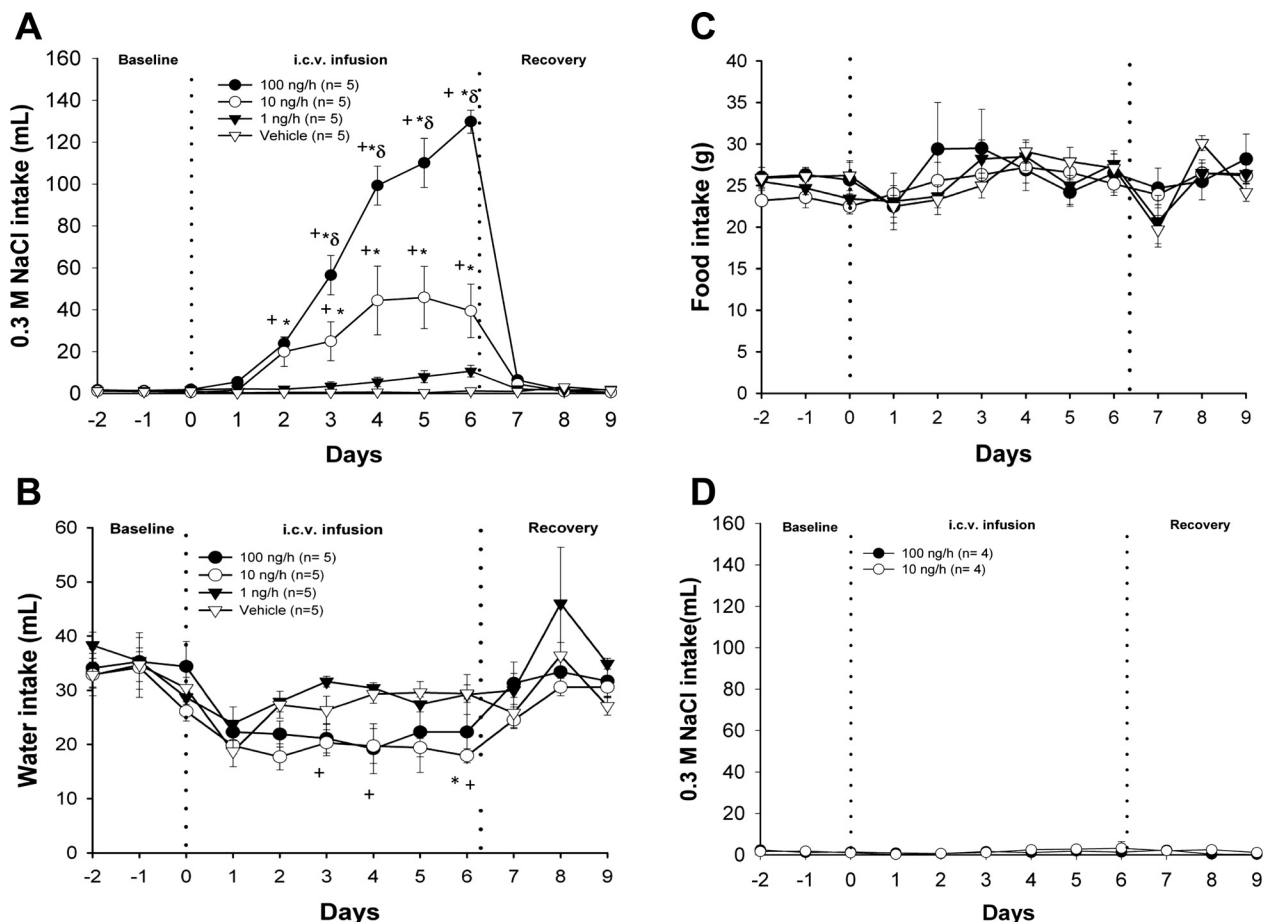


Fig. 1. Intakes of hypertonic NaCl, water, and food by rats that received long-term infusion of aldosterone into the brain ventricles with a syringe pump. Daily intake of 0.3 M NaCl (A), daily water intake (B), and daily food intake (C) by rats that received aldosterone infusion into the 4th ventricle. D: daily intake of 0.3 M NaCl by rats that received aldosterone infusion into the lateral ventricle (LV). Error bars show means \pm SE. *Significantly different from vehicle ($P < 0.05$). +/Significantly different from aldosterone 1 ng/h. δ Significantly different from aldosterone 10 ng/h.

Table 1. *Body weight immediately before and after 6 days of intracerebroventricular infusion of aldosterone*

	Initial Weight, g	Final Weight, g
4th Ventricle infusion		
Vehicle (5)	347 ± 11	346 ± 10
Aldo 1 ng/h (5)	343 ± 11	356 ± 9
Aldo 10 ng/h (5)	330 ± 15	325 ± 16
Aldo 100 ng/h (5)	320 ± 9	313 ± 12
LV infusion		
Aldo 10 ng/h (4)	296 ± 6	310 ± 6
Aldo 100 ng/h (4)	290 ± 4	298 ± 8

Data are expressed as means ± SE. Values in parentheses show the number of animals. Aldo, aldosterone; LV, lateral ventricle.

from a 30-gauge needle. The injector, when fully inserted, protruded 2 mm (4th V) or 1.5 mm (LV) beyond the tip of the guide cannula. Injections were 2 µl in about 30 s.

Drugs Injected

Aldosterone (Sigma, St. Louis, MO) was prepared at concentrations of 0.5, 5, 50, and 250 µg/ml. RU 28318 (MR antagonist, Tocris Bioscience, Ellisville, MO) was prepared at a concentration of 100 ng/2 µl (10, 11, 31, 47). Solutions of aldosterone were made fresh from a stock solution of 20 mM of aldosterone maintained in a refrigerator. Vehicle for aldosterone and RU 28318 was 1% ethanol in 0.9% NaCl.

Measurement of Intakes of 0.3 M NaCl, Water, and Food

In experiments with chronic infusions, rats had free access to water and 0.3 M NaCl in burettes with 1-ml divisions that were fitted with metal drinking spouts. A preweighed amount of regular pelleted chow was made available. Spilled chow was collected and considered in the calculation of food intake. Intakes of water, 0.3 M NaCl, and food were measured each 24 h.

For measurement of intakes in 2-h intake tests, water and 0.3 M NaCl were presented in burettes with 0.1-ml division fitted with metal drinking spouts. Intakes of water and 0.3 M NaCl were measured at 15, 30, 60, 90, and 120 min after the access to the fluids.

Histology

At the end of experiments, rats were deeply anesthetized with a lethal intraperitoneal dose of urethane (Sigma, St. Louis, MO) and then received an injection of Evan's blue dye (100 nl of a 2% solution) into the 4th V or LV. Rats were perfused transcardially with 10% formalin. The brain was removed, stored in buffered formalin for at least 2 days, and cut in 50-µm coronal sections with a microtome. Slices were stained by the Giemsa method and analyzed by light microscopy. Only rats with clear spread of dye in the LV or 4th V were used for data analysis.

Statistical Analysis

The results are shown as means ± SE. Intakes in tests with long-term infusion of aldosterone were compared by two-way ANOVA, with factors drug dose and time. Intakes after 9 days of low-sodium diet similarly were compared by two-way ANOVA. Intakes after treatment with furosemide followed by 24 h without access to sodium were compared by two-way repeated-measures ANOVA. Significant ANOVAs were followed by Tukey's post hoc test. Body weights before and after the treatments were compared with paired *t*-tests. Statistical analyses were performed with SigmaStat 2.0 software (Jandel, Point Richmond, CA). The level of significance was set at *P* < 0.05.

Experimental Protocols

Hypertonic NaCl and water intake by rats treated with chronic infusion of aldosterone in the 4th or LV. Infusions were made either with a syringe pump or with an implantable osmotic minipump. In experiments with syringe pump infusions, daily ingestion of food, water, and 0.3 M NaCl were recorded for a period of 12 days that included a 3-day preinfusion control period, a 6-day period of continuous infusion, and a 3-day postinfusion recovery period. Infusions into the 4th V contained either vehicle (2 µl/h) or 1, 10, or 100 ng/h of aldosterone (*n* = 5/dose). Infusions into the LV contained either vehicle or 10 or 100 ng/h aldosterone (*n* = 4/dose). Body weight was measured just before the start of the infusion and immediately after the end of the infusion. Each rat received only one type of infusion.

In experiments with osmotic minipump infusions, daily intakes of water and 0.3 M NaCl were recorded for a period of 13 days that included a 3-day preinfusion control period, 7 days of infusion, and a 3-day postinfusion recovery period. Infusions into the 4th V contained 100 ng/h of aldosterone (*n* = 5). Infusions in the LV contained 100 or 500 ng/h of aldosterone (*n* = 5 or 6). Each rat received only one type of infusion.

Sodium depletion-induced hypertonic NaCl intake by rats treated with a bolus injection of aldosterone antagonist into the 4th V or LV. Rats were depleted of sodium with two injections of the diuretic furosemide (Hypolabor, Sabará, MG, Brazil), which were each 10 mg/kg body wt sc, made with a 30-min interval of each other. Then, the rats were kept with access only to water and a low-sodium diet for the next 24 h. After this period, the rats received a single injection of vehicle or RU 28318 (100 ng/2 µl) into the 4th V (*n* = 6/group) or into the LV (*n* = 6/group). Two hours after the injection, access was allowed to graduated tubes containing water and 0.3 M NaCl. Water and 0.3 M NaCl intakes were measured at 15, 30, 60, 90, and 120 min. The rats were sodium-depleted once a week during 3 wk. The first sodium depletion was used only for training, rats received no central injection, and data were not analyzed. On the second and the third depletion, rats received the drug and the vehicle in random order.

Low-sodium diet-induced hypertonic NaCl intake by rats treated with bolus injection of aldosterone antagonist into the 4th V. Rats had free access to low-sodium chow (0.005% Na⁺; Prag Soluções Bio-ciências, Jaú, SP, Brazil) and distilled water for 9 days. After this

Table 2. *Water intake during 6 days of infusion of aldosterone into the LV*

	Days					
	1	2	3	4	5	6
Vehicle	33.3 ± 2.3	30.3 ± 2	35.5 ± 2.2	29.7 ± 1.1	32.1 ± 1	29 ± 0.5
Aldo, 10 ng/h	27.8 ± 2.2	25.5 ± 1.9	28.7 ± 1.9	27.1 ± 2.2	30.1 ± 2.5	35.2 ± 5.1
Vehicle	30.2 ± 1.4	30.1 ± 3.4	26.7 ± 1.6	26.2 ± 3.4	25.4 ± 3.7	26 ± 4
Aldo, 100 ng/h	24.6 ± 3.5	26.2 ± 3.5	26.2 ± 2.5	25.4 ± 2.9	24.4 ± 3.5	22.9 ± 3.1

Data are expressed as means ± SE; *n* = 4 rats in each group.

Table 3. Food intake during 6 days of infusion of aldosterone into the LV

	Days					
	1	2	3	4	5	6
Vehicle	22 ± 1.2	23.1 ± 1.1	27.7 ± 2.6	22.6 ± 0.3	22.7 ± 2.1	24.7 ± 0.9
Aldo, 10 ng/h	27 ± 2.6	24.3 ± 1.9	27.1 ± 2.8	27.5 ± 2.2	29.6 ± 2.5	25.9 ± 1.4
Vehicle	21.3 ± 1.2	23.5 ± 3.4	21 ± 1.7	21.4 ± 2.6	21.4 ± 2.4	21.9 ± 1.8
Aldo, 100 ng/h	22.1 ± 3.1	23 ± 3.3	21.4 ± 4	24.9 ± 2.3	23.9 ± 1.4	22.4 ± 1.9

Data are expressed as means ± SE; $n = 4$ rats in each group.

period, the rats randomly received an injection of vehicle ($n = 4$) or RU 28318 (100 ng/2 μ l, $n = 5$) into the 4th V. Two hours after the injection into the 4th V, access was allowed to graduated tubes containing distilled water and 0.3 M NaCl. Water and 0.3 M NaCl intake was measured at 15, 30, 60, 90, and 120 min.

RESULTS

Intakes of hypertonic NaCl, water, and food by rats treated with chronic infusion of aldosterone into the 4th and LV. In experiments with tethered rats (syringe infusion), infusion of aldosterone into the 4th V caused a dose-dependent increase in daily intake of 0.3 M NaCl, with significant differences between treatments [$F(3, 192) = 126.89$; $P < 0.001$] (Fig. 1A). The highest dose of aldosterone (100 ng/h) induced strong daily ingestion of 0.3 M NaCl, with intakes reaching 130 ± 6 ml/24 h on the 6th day of infusion (Fig. 1A). After the end of the infusions, NaCl intake rapidly (within 24 h) returned to baseline levels (Fig. 1A). This high dose of aldosterone was significantly more effective than lower doses (1 or 10 ng/h) from the 3rd to 6th day of infusion. Infusion of 10 ng/h induced 0.3 M NaCl intakes of up to 46 ± 15 ml/24 h on 5th day of infusion. This dose was significantly more effective than the lowest dose (1 ng/h) from the 4th to 6th day of infusion. The 1 ng/h dose of aldosterone produced only small 0.3 M NaCl intakes (up to 9 ± 3 ml/24 h on the 6th day of infusion, Fig. 1A), but this was not significantly different from vehicle.

Water intake fell with aldosterone infusions in the 4th V, with a significant difference between the 10 ng/h group and vehicle on day 6 of infusion [$F(3, 192) = 11.15$; $P < 0.001$] (Fig. 1B). On the 3rd and 6th day of infusion, this dose of aldosterone also reduced water intake compared with the lowest dose of aldosterone (1 ng/h). The highest dose of aldosterone (100 ng/h) reduced daily water intake compared with the lowest dose of aldosterone (1 ng/h) on the 4th day of infusion. Aldosterone (1, 10, or 100 ng/h) did not change daily food intake (Fig. 1C) or body weight (Table 1).

In contrast with 4th V infusions, LV infusions (10 or 100 ng/h of aldosterone for 6 days) failed to stimulate daily intake of 0.3 M NaCl [$F(2, 108) = 1.05$; $P > 0.05$] (Fig. 1D). Infusion of aldosterone into the LV also did not alter body weight and daily water or food intake (Tables 1, 2, and 3).

Infusion of aldosterone (100 ng/h) into the 4th V with osmotic minipumps induced 0.3 M NaCl intakes of up to 50.7 ± 5.9 ml/24 h on the 7th day of infusion, which differed from the baseline period from the 4th to 7th day of infusion [$F(2, 128) = 73.97$; $P < 0.0001$] (Fig. 2A). After the end of the infusions, 0.3 M NaCl intake rapidly (within 24 h) returned to baseline levels (Fig. 2A). This 4th V infusion did not change daily water intake.

Infusion of aldosterone (100 and 500 ng/h) into the LV with osmotic minipumps did not affect daily intake of 0.3 M NaCl (Fig. 2A) or water (Fig. 2B).

Sodium depletion-induced hypertonic NaCl intake by rats treated with bolus injection of aldosterone antagonist into the 4th V and LV. Injection of the aldosterone antagonist RU 28318 (100 ng/2 μ l) into the 4th V reduced 0.3 M NaCl intake induced by 24 h of sodium depletion (furosemide followed by

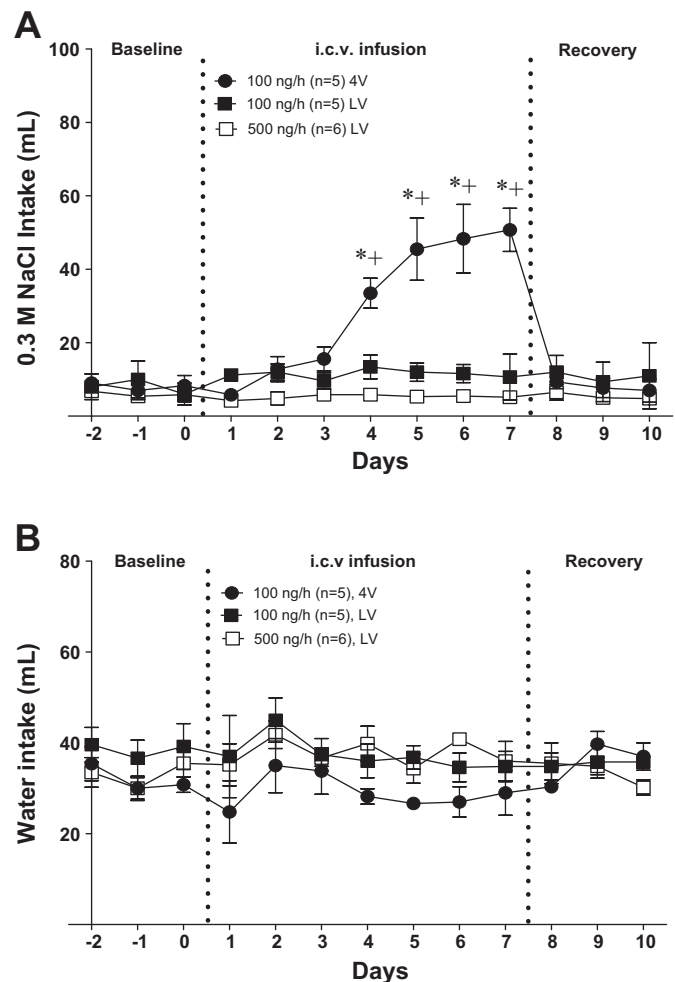


Fig. 2. Intakes of hypertonic NaCl and water by rats that received long-term infusion of aldosterone into the brain ventricles with osmotic minipumps. Daily intake of 0.3 M NaCl (A) and daily water intake (B) by rats that received infusions of aldosterone into the LV and fourth ventricle (4th V). Error bars show means ± SE. *Significantly different from the intake in the baseline or recovery period in the same group ($P < 0.05$). +Significantly different from aldosterone infused into the LV ($P < 0.05$).

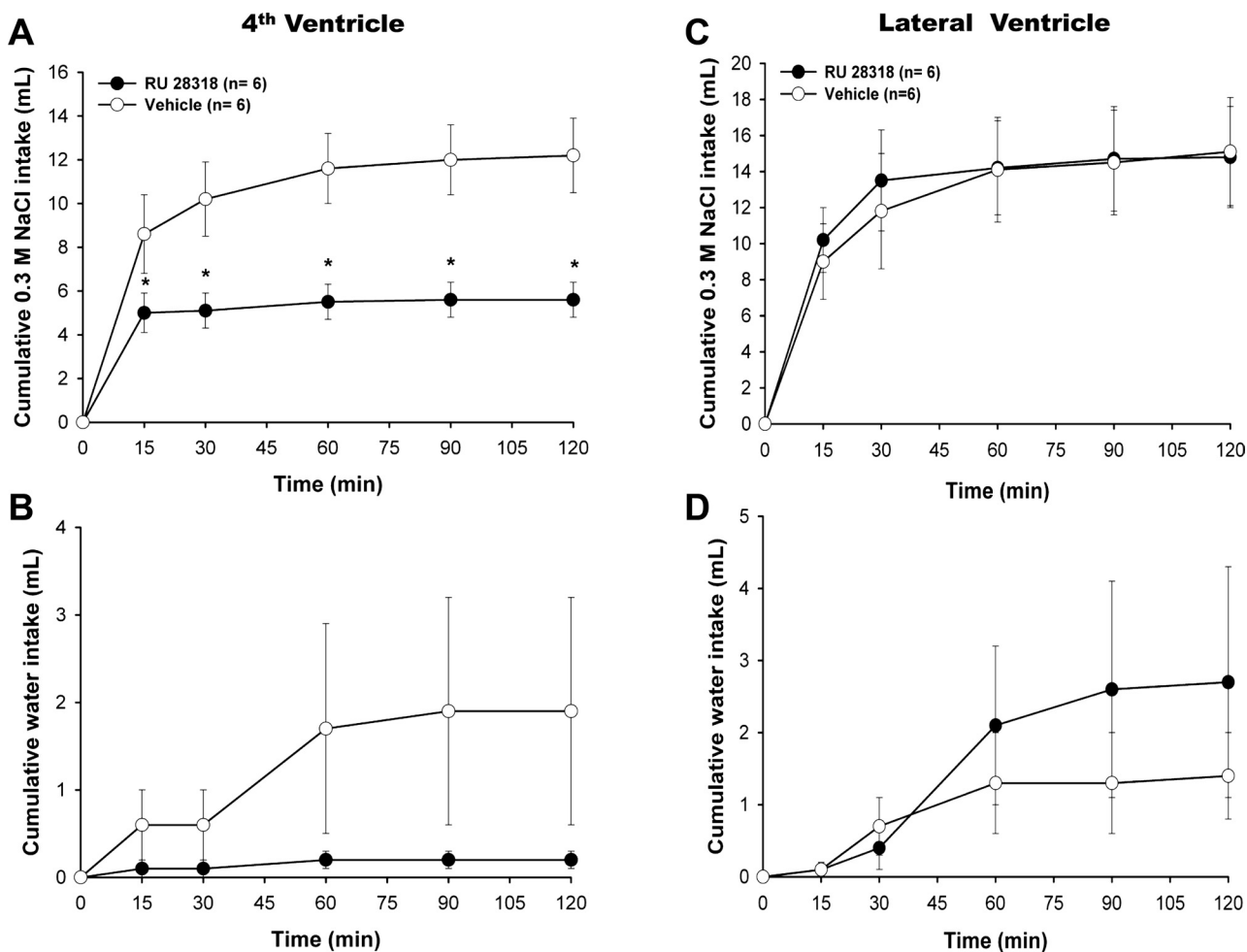


Fig. 3. Intake of water and 0.3 M NaCl induced by 24 h of sodium depletion in rats treated with bolus injection of the mineralocorticoid receptor antagonist RU 28318 (100 ng at $t = -2$ h) into the brain ventricles. A and B: intake of 0.3 M NaCl and water by rats that received injection into the 4th V. C and D: intake of 0.3 M NaCl and water by rats that received injection into the LV. Error bars show means \pm SE. *Significantly different from vehicle.

24 h without access to sodium) (5.6 ± 0.8 ml/2 h vs. vehicle: 12 ± 1.7 ml/2 h) [$F(1, 5) = 22.54$; $P < 0.05$] (Fig. 3A). Water intake was not affected by RU 28318 [$F(1, 5) = 1.72$; $P > 0.05$] (Fig. 3B). Injection in the LV of this dose of RU 28318 did not affect sodium depletion-induced intake of 0.3 M NaCl (14.8 ± 2.8 vs. vehicle: 15.1 ± 3 ml/2 h) [$F(1, 5) = 0.19$; $P > 0.05$] and water [$F(1, 5) = 0.56$; $P > 0.05$] (Fig. 3, C and D).

Low-sodium diet-induced hypertonic NaCl intake by rats treated with bolus injection of aldosterone antagonist into the 4th V. Injection of RU 28318 (100 ng/2 μ l) into the 4th V abolished 0.3 M NaCl intake induced by 9 days of low-sodium diet (1.2 ± 0.6 vs. vehicle: 9.5 ± 2.6 ml/2 h) [$F(1, 35) = 47.69$; $P < 0.001$] (Fig. 4A). Sodium-deprived rats drank little water, but water intake was further reduced by RU 28318 (0.1 ± 0.1 vs. vehicle: 0.5 ± 0.1 ml/2 h) [$F(1, 35) = 20.86$; $P < 0.001$] (Fig. 4B).

DISCUSSION

The present results show that chronic infusion of aldosterone into the 4th V induced strong daily ingestion of hypertonic NaCl without changing food intake or body weight, whereas the injection of the specific MR antagonist RU 28318 into the

4th V reduced NaCl intake induced by either 24 h of sodium depletion or low-sodium diet. Water intake was not consistently affected by these treatments. These results suggest that mineralocorticoid mechanisms in the hindbrain are important for the control of sodium intake.

Although both methods used to infuse aldosterone into the 4th ventricle induced a strong sodium appetite, syringe infusions produced larger intakes and possibly a faster onset of sodium appetite. With osmotic minipumps, infusions were delayed by the time required for the drug to pass the infusion tubing and injection cannula, whereas syringe infusions did not have this delay. Also, drug degradation may be less of a factor with syringe infusions, as infusion solutions were prepared fresh every day.

Whereas the administration of aldosterone or MR antagonist into the 4th V caused large changes in hypertonic NaCl intake, the administration in the LV had no effect. A similar difference between effects of infusion of aldosterone into the LV and more caudally has been reported for sodium excretion. Aldosterone induces natriuresis when infused in the subcommissural organ region, close to the aqueduct, but not when infused in the LV (10, 11). In contrast to our finding that a bolus injection of a MR antagonist into the LV did not reduce sodium intake, it

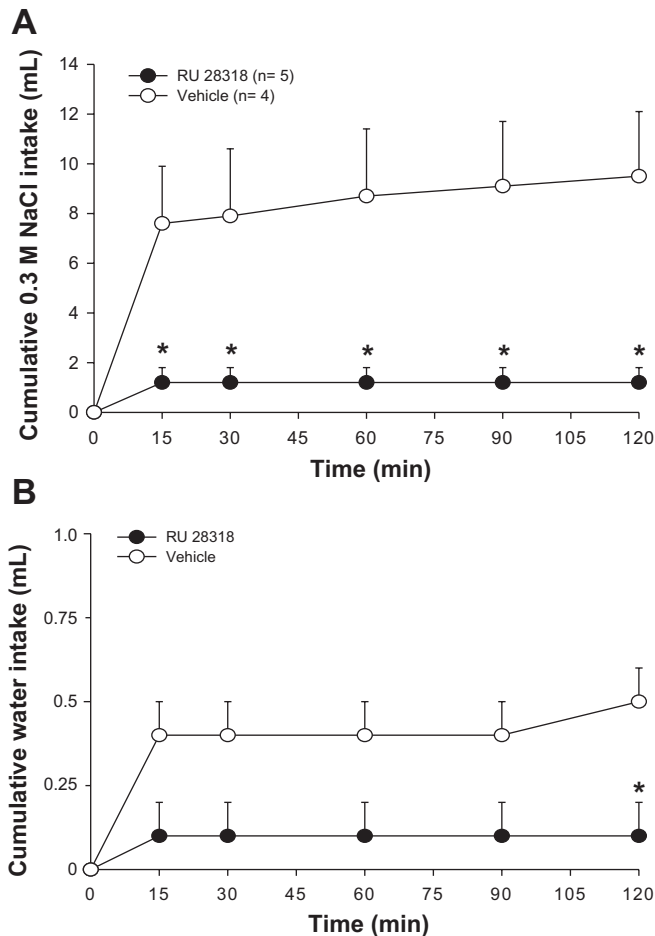


Fig. 4. Intake of water and 0.3 M NaCl induced by 9 days of a low-sodium diet in rats treated with bolus injection of RU 28318 into the 4th V. A: intake of 0.3 M NaCl. B: water intake. Error bars show means \pm SE. *Significantly different from vehicle.

has been reported that continuous infusion of MR antagonists (spironolactone or RU 28318) into the LV for one or more days reduced sodium intake in rats (16, 47, 53). The different effects of LV and 4th V infusions might be related to the ease with which drug reaches the sites involved in the control of sodium intake. It is well known that forebrain areas, such as the amygdala are involved in aldosterone-induced sodium intake (16, 45, 46, 47, 53). However, the distance between the amygdala and the LV is significant, and this may prevent an easy access to the amygdala by drugs injected in the LV. In contrast, the NTS, and, particularly, the HSD2 neurons in this area are located close to the 4th V and might be easily affected by drugs injected into the 4th V (20, 21). Although the present results suggest that hindbrain mineralocorticoid mechanisms are involved in the control of sodium intake, they are not incompatible with a role for mineralocorticoid receptors in the amygdala.

Earlier studies suggested that the amygdala is a main central site activated by aldosterone to induce sodium appetite (37, 45, 46, 60). Salt appetite is elicited in normohydrated and satiated rats immediately after the injection of aldosterone or DOCA into the amygdala and the administration of antisense oligodeoxynucleotides against MR into the amygdala reduced DOCA-

induced sodium intake (45, 46). More recent neuroanatomical studies have shown aldosterone-sensitive neurons (HSD2 neurons) in the NTS that are activated in sodium-depleted rats and deactivated by hypertonic NaCl ingestion, suggesting that these neurons play a role in body sodium homeostasis (20, 21, 22). These neurons are located in the medial border of the NTS close to the 4th V (20, 21, 22), and therefore, they might be easily affected by aldosterone and MR antagonists infused into the 4th V. Therefore, the present functional results complement previous neuroanatomical studies (20, 21, 22, 23), suggesting that hindbrain MR receptors located close to the 4th V (possibly on HSD2 neurons in the NTS) are important for aldosterone-induced sodium intake.

Aldosterone infused into the 4th V may produce natriuretic responses by directly activating facilitatory mechanisms or by modulating inhibitory mechanisms like the lateral parabrachial nucleus (LPBN) mechanisms that are thought to depend on NTS signals (4, 34, 35). It seems less likely that increased intake is a consequence of renal sodium loss, although chronic central infusion of aldosterone into the region of the subcommissural organ, close to the aqueduct, is known to induce natriuresis (10, 11). In addition, the acute inhibition of sodium intake by injection of MR antagonist into the 4th V suggests that the activation of sodium intake by hindbrain MR does not depend on natriuretic effects of aldosterone acting on the brain stem.

Although the infusion of aldosterone in the 4th V induced intake of large volumes of hypertonic NaCl, it failed to increase water intake. In fact, infusion of 10 ng/h of aldosterone tended to reduce water intake. The mechanism of this reduced water intake is not yet clear. As discussed above, central infusion of aldosterone may produce natriuresis (10, 11), and reduced plasma sodium may facilitate sodium intake and inhibit water intake. Intake of hypertonic NaCl increases plasma sodium, and this usually drives rats to ingest water. Sodium-depleted rats ingest a small (less than 3 ml in a 2-h test) amount of water when they have access to 0.3 M NaCl (49, 50, 51, 52). As this water intake is small and highly variable, differences on water intake in these tests are usually not statistically significant, as in the present study.

A synergism between aldosterone and ANG II in the control of sodium appetite has been suggested, and the complete suppression of sodium depletion-induced sodium intake requires a combination of blockade of central MR and ANG II action (13, 15, 47, 50). In the present study, the injection of a MR antagonist into the 4th V almost abolished sodium intake induced by a low-sodium diet, whereas sodium intake induced by 24 h of sodium depletion fell by only 50%, suggesting that sodium intake after 9 days of dietary sodium depletion depends more on mineralocorticoid mechanisms. Sodium appetite in rats treated with furosemide is known to depend on increased circulating ANG II (9, 17, 44) and, therefore, sodium appetite remaining in these rats after the blockade of hindbrain MR might be due to ANG II or possibly to MR receptors in the forebrain that may still act in a synergism with ANG II (15, 50, 54).

The actions of mineralocorticoids in the brain involve both rapid nongenomic mechanisms dependent on cell membrane MRs and slow genomic mechanisms that depend on cytosolic MRs. Both mechanisms are thought to be involved in the control of sodium intake (46, 47, 56). The genomic mecha-

nisms and the synergism between aldosterone and ANG II might explain why a strong ingestion of hypertonic NaCl arises only several hours after sodium depletion, like the ingestion of sodium by 24-h sodium-depleted rats (44). The ingestion of small volumes of 0.25 M NaCl (2 to 3 ml in 30 min) induced by aldosterone injection into the amygdala (46) and the reduction of sodium appetite 2 h after injection of RU 28318 into the 4th V (present results) suggest the involvement of nongenomic mechanisms. The daily increase in sodium intake during 6 days of aldosterone infusion into the 4th V might be the result of cumulative activation of the genomic mechanisms. However, sodium intake returned to control levels shortly after stopping aldosterone infusion, which would not be expected if genomic mechanisms were involved in this behavior.

In conclusion, the present study shows that infusion of aldosterone into the 4th V induces strong daily ingestion of sodium, whereas a MR antagonist injected into the 4th V reduces sodium intake induced by either 24 h of sodium depletion or chronic low-sodium diet. The results suggest that the MR receptors located in the hindbrain close to the 4th V (possibly HSD2 neurons in the NTS) are part of the mechanisms involved in the natriorexigenic response produced by aldosterone.

Perspectives and Significance

Important facilitatory and inhibitory mechanisms involved in the control of sodium appetite have been described in the forebrain and hindbrain. The present study suggests that important mineralocorticoid mechanisms involved in the control of sodium intake are present in the hindbrain. Interactions between forebrain and hindbrain mechanisms involved in the control of sodium intake probably exist, and an understanding of how the hindbrain interacts with the forebrain in the control of salt appetite and also the relative importance of forebrain and hindbrain MRs in the control of sodium intake are the challenges for the future research. Activation of MR may also increase sympathetic activity in animals and in hypertensive patients (5, 18, 24, 25, 26, 27, 58). The NTS that harbors the HSD2 neurons activated by aldosterone is an important site for the control of sympathetic nerve activity. Therefore, future studies might investigate whether the intense sodium appetite produced by the action of aldosterone on the hindbrain is accompanied by aldosterone-induced sympathetic activation. This combination of effects may make aldosterone a particularly effective hypertensive factor in the case of unbalanced secretion of this hormone.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.F. and E.C. conception and design of research; S.F., M.B., N.B.N., and E.C. performed experiments; S.F., M.B., N.B.N., G.H.M.S., J.V.M., and E.C. analyzed data; S.F., J.V.M., and E.C. interpreted results of experiments; S.F., M.B., and E.C. prepared figures; S.F., G.H.M.S., and E.C.

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REFERENCES

- Andrade-Franzé GM, Andrade CA, De Luca LA Jr, De Paula PM, Colombari DS, Menani JV. Lesions in central amygdala impair sodium intake induced by the blockade of the lateral parabrachial nucleus. *Brain Res* 1332: 57–64, 2010.
- Antunes-Rodrigues J, de Castro M, Elias LLK, Valença MM, McCann SM. Neuroendocrine control of body fluid metabolism. *Physiol Rev* 84: 169–208, 2004.
- Blair-West JR, Coghlan JP, Denton DA, Nelson JF, Orchard E, Scoggins BA, Wright RD, Myers K, Junqueira CL. Physiological, morphological and behavioural adaptation to a sodium deficient environment by wild native Australian and introduced species of animals. *Nature* 217: 922–928, 1968.
- Colombari DSA, Menani JV, Johnson AK. Forebrain angiotensin type 1 receptors and parabrachial serotonin in the control of NaCl and water intake. *Am J Physiol Regul Integr Comp Physiol* 271: R1470–R1476, 1996.
- Conlin PR. Interactions of high salt intake and the response of the cardiovascular system to aldosterone. *Cardiol Rev* 13: 118–124, 2005.
- Contreras RJ, Stetson PW. Changes in salt appetite after lesions of the area postrema and the nucleus of the solitary tract in rats. *Brain Res* 211: 355–366, 1981.
- Curtis KS, Huang W, Sved AF, Verbalis JG, Stricker EM. Impaired osmoregulatory responses in rats with area postrema lesions. *Am J Physiol Regul Integr Comp Physiol* 277: R209–R219, 1999.
- De Luca LA Jr, Galaverna O, Schulkin J, Yao SZ, Epstein AN. The anteroventral wall of the third ventricle and the angiotensinergic component of need-induced sodium intake in the rat. *Brain Res Bull* 28: 73–87, 1992.
- Douglas J, Catt KJ. Regulation of angiotensin II receptors in the rat adrenal cortex by dietary electrolytes. *J Clin Invest* 58: 834–843, 1976.
- Dundore RL, Wurlpel JND, Balaban CD, Keil LC, Severs WB. Central effects of aldosterone infused into the rat subcommissural organ region. *Neurosci Res* 1: 341–351, 1984.
- Dundore RL, Wurlpel JND, Balaban CD, Harrison TS, Keil LC, Seaton JF, Severs WB. Site-dependent central effects of aldosterone in rats. *Brain Res* 401: 122–131, 1987.
- Edwards GL, Beltz TG, Power JD, Johnson AK. Rapid-onset “need-free” sodium appetite after lesions of the dorsomedial medulla. *Am J Physiol Regul Integr Comp Physiol* 264: R1242–R1247, 1993.
- Epstein AN. Mineralocorticoids and cerebral angiotensin may act together to produce sodium appetite. *Peptides* 3: 493–494, 1982.
- Epstein AN, Massi M. Salt appetite in the pigeon in response to pharmacological treatments. *J Physiol* 393: 555–568, 1987.
- Fluharty SJ, Epstein AN. Sodium appetite elicited by intracerebroventricular infusion of angiotensin II in the rat: II. Synergistic interaction with systemic mineralocorticoids. *Behav Neurosci* 97: 746–758, 1983.
- Francis J, Weiss RM, Wei SG, Johnson AK, Beltz TG, Zimmerman K, Felder RB. Central mineralocorticoid receptor blockade improves volume regulation and reduces sympathetic drive in heart failure. *Am J Physiol Heart Circ Physiol* 281: H2241–H2251, 2001.
- Fregly MJ, Rowland NE. Role of renin-angiotensin-aldosterone system in NaCl appetite of rats. *Am J Physiol Regul Integr Comp Physiol* 248: R1–R11, 1985.
- Fujita T. Aldosterone in salt-sensitive hypertension and metabolic syndrome. *J Mol Med* 86: 729–734, 2008.
- Galaverna O, De Luca LA Jr, Schulkin J, Yao SZ, Epstein AN. Deficits in NaCl ingestion after damage to the central nucleus of amygdala in the rat. *Brain Res Bull* 28: 89–98, 1992.
- Geerling JC, Engeland WC, Kawata M, Loewy AD. Aldosterone target neurons in the nucleus tractus solitarius drive sodium appetite. *J Neurosci* 26: 411–417, 2006.
- Geerling JC, Kawata M, Loewy AD. Aldosterone-sensitive neurons in the rat central nervous system. *J Comp Neurol* 494: 515–527, 2006.
- Geerling JC, Loewy AD. Aldosterone-sensitive NTS neurons are inhibited by saline ingestion during chronic mineralocorticoid treatment. *Brain Res* 1115: 54–64, 2006.
- Geerling JC, Loewy AD. Sodium depletion activates the aldosterone-sensitive neurons in the NTS independently of thirst. *Am J Physiol Regul Integr Comp Physiol* 292: R1338–R1348, 2007.

24. Gomez-Sanchez EP. Intracerebroventricular infusion of aldosterone induces hypertension in rats. *Endocrinology* 118: 819–823, 1986.
25. Huang BS, Wang H, Leenen FHH. Chronic central infusion of aldosterone leads to sympathetic hyperactivity and hypertension in Dahl S but not Dahl R rats. *Am J Physiol Heart Circ Physiol* 288: H517–H524, 2005.
26. Huang BS, White RA, Ahmad M, Jeng AY, Leenen FHH. Central infusion of aldosterone synthase inhibitor prevents sympathetic hyperactivity and hypertension by central Na⁺ in Wistar rats. *Am J Physiol Regul Integr Comp Physiol* 295: R166–R172, 2008.
27. Huang BS, White RA, Jeng AY, Leenen FHH. Role of central nervous system aldosterone synthase and mineralocorticoid receptors in salt-induced hypertension in Dahl salt-sensitive rats. *Am J Physiol Regul Integr Comp Physiol* 296: R994–R1000, 2009.
28. Hyde TM, Miselis RR. Area postrema and adjacent nucleus of the solitary tract in water and sodium balance. *Am J Physiol Regul Integr Comp Physiol* 247: R173–R182, 1984.
29. Johnson AK, Thunhorst RL. The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. *Front Neuroendocrinol* 18: 292–353, 1997.
30. Johnson AK, Thunhorst RL. The neuroendocrinology, neurochemistry and molecular biology of thirst and salt appetite. In: *Handbook of Neurochemistry and Molecular Neurobiology: Behavioral Neurochemistry, Neuroendocrinology and Molecular Neurobiology*, edited by Lajtha A and Blaustein JD. New York, NY: Springer, 2007.
31. Kim PJ, Cole MA, Kalman BA, Spencer RL. Evaluation of RU 28318 and RU 40555 as selective mineralocorticoid receptor and glucocorticoid receptor antagonists, respectively: Receptor measures and functional studies. *J Steroid Biochem Molec Biol* 67: 213–222, 1998.
32. Kochli A, Tenenbaum-Rakover Y, Leshem M. Increased salt appetite in patients with congenital adrenal hyperplasia 21-hydroxylase deficiency. *Am J Physiol Regul Integr Comp Physiol* 288: R1673–R1681, 2005.
33. Ma LY, McEwen BS, Sakai RR, Schulkin J. Glucocorticoids facilitate mineralocorticoid-induced sodium intake in the rat. *Horm Behav* 27: 240–250, 1993.
34. Menani JV, Colombari DSA, Beltz TG, Thunhorst RL, Johnson AK. Salt appetite: Interaction of forebrain angiotensinergic and hindbrain serotonergic mechanisms. *Brain Res* 801: 29–35, 1998.
35. Menani JV, Thunhorst RL, Johnson AK. Lateral parabrachial nucleus and serotonergic mechanisms in the control of salt appetite in rats. *Am J Physiol Regul Integr Comp Physiol* 270: R162–R168, 1996.
36. Náray-Fejes-Tóth A., Fejes-Tóth G. Subcellular localization of the type 2 11 β -hydroxysteroid dehydrogenase. *J Biol Chem* 271: 15436–15442, 1996.
37. Nitabach MN, Schulkin J, Epstein AN. The medial amygdala is part of a mineralocorticoid-sensitive circuit controlling NaCl intake in the rat. *Behav Brain Res* 35: 127–134, 1989.
38. Odermatt A, Arnold P, Frey FJ. The intracellular localization of the mineralocorticoid receptor is regulated by 11 β -hydroxysteroid dehydrogenase type 2. *J Biol Chem* 276: 28484–28492, 2001.
39. Odermatt Arnold P A, Stauffer A, Frey BM, Frey FJ. The N-terminal anchor sequences of 11 β -hydroxysteroid dehydrogenase determine their orientation in the endoplasmic reticulum membrane. *J Biol Chem* 274: 28762–28770, 1999.
40. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. New York, NY: Academic, 1986.
41. Reilly JJ, Maki R, Nardozi J, Schulkin J. The effects of lesions of the bed nucleus of the stria terminalis on sodium appetite. *Acta Neurobiol Exp (Warsz)* 54: 253–257, 1994.
42. Rice KK, Richter CP. Increased sodium chloride and water intake of normal rats treated with desoxycorticosterone acetate. *Endocrinology* 33: 106–115, 1943.
43. Richter CP. Increased salt appetite in adrenalectomized rats. *Am J Physiol* 115: 155–161, 1936.
44. Rowland NE, Morian KR. Roles of aldosterone and angiotensin in maturation of sodium appetite in furosemide-treated rats. *Am J Physiol Regul Integr Comp Physiol* 276: R1453–R1470, 1999.
45. Sakai RR, Ma LY, Zhang DM, McEwen BS, Fluharty SJ. Intracerebral administration of mineralocorticoid receptor antisense oligonucleotides attenuate adrenal steroid-induced salt appetite in rats. *Neuroendocrinology* 64: 425–429, 1996.
46. Sakai RR, McEwen BS, Fluharty SJ, Ma LY. The amygdala: Site of genomic and non-genomic arousal of aldosterone-induced sodium intake. *Kidney Int* 57: 1337–1345, 2000.
47. Sakai RR, Nicolaidis S, Epstein AN. Salt appetite is suppressed by interference with angiotensin II and aldosterone. *Am J Physiol Regul Integr Comp Physiol* 251: R762–R768, 1986.
48. Scalera G, Spector AC, Norgren. Excitotoxic lesions of the parabrachial nuclei prevent conditioned taste aversions and sodium appetite in rats. *Behav Neurosci* 109: 997–1008, 1995.
49. Schulkin J, Marini J, Epstein AN. A role for the medial region of the amygdala in mineralocorticoid-induced salt hunger. *Behav Neurosci* 103: 179–185, 1989.
50. Shade RE, Blair-West JR, Carey KD, Madden LJ, Weisinger RS, Denton DA. Synergy between angiotensin and aldosterone in evoking sodium appetite in baboons. *Am J Physiol Regul Integr Comp Physiol* 283: R1070–R1078, 2002.
51. Stellar E, Hyman R, Samet S. Gastric factors controlling water-and salt-solution-drinking. *J Comp Physiol Psychol* 47: 220–226, 1954.
52. Stricker EM, Bushey MA, Hoffmann ML, McGhee M, Cason AM, Smith JC. Inhibition of NaCl appetite when DOCA-treated rats drink saline. *Am J Physiol Regul Integr Comp Physiol* 292: R652–R662, 2007.
53. Sullivan MJ, Hasser EM, Moffitt JA, Bruno SB, Cunningham JT. Rats exhibit aldosterone-dependent sodium appetite during 24 h hindlimb unloading. *J Physiol* 557: 661–670, 2004.
54. Xue B, Beltz TG, Yu Y, Gomez-Sanchez CE, Hay M, Johnson AK. Central interactions of aldosterone and angiotensin II in aldosterone and angiotensin-induced hypertension. *Am J Physiol Heart Circ Physiol* 300: H555–H564, 2011.
55. Wang T, Edwards GL. Differential effects of dorsomedial medulla lesion size on ingestive behavior in rats. *Am J Physiol Regul Integr Comp Physiol* 273: R1299–R1308, 1997.
56. Wehling M, Christ M, Theisen K. Membrane receptors for aldosterone: a novel pathway for mineralocorticoid action. *Am J Physiol Endocrinol Metab* 263: E974–E979, 1992.
57. Wilkins L, Richter CP. A great craving for salt by a child with cortico-adrenal insufficiency. *JAMA* 114: 866–868, 1940.
58. Wray DW, Supiano MA. Impact of aldosterone receptor blockade compared with thiazide therapy on sympathetic nervous system function in geriatric hypertension. *Hypertension* 55: 1217–1223, 2010.
59. Zardetto-Smith AM, Beltz TG, Johnson AK. Role of the central nucleus of the amygdala and the bed nucleus of the stria terminalis in experimentally-induced salt appetite. *Brain Res* 645: 123–134, 1994.
60. Zhang DM, Epstein AN, Schulkin J. Medial region of the amygdala: involvement in adrenal-steroid-induced salt appetite. *Brain Res* 600: 20–26, 1993.